

BENEFITS AND COSTS OF DEFENSE IN A NEOTROPICAL SHRUB¹

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Abstract. Benefits and costs are central to optimality theories of plant defense. Benefit is the gain in fitness to reducing herbivory and cost is the loss in fitness to committing resources to defense. We evaluate the benefits and costs of defense in a neotropical shrub, *Psychotria horizontalis*. Plants were either exposed to herbivores or protected within a cage of fine mesh in three gardens planted in large light gaps on Barro Colorado Island, Panama. Two cuttings of each of 35 clones were paired based on initial masses and assigned randomly to a garden and to either the exposed or protected treatment. Annual rates of herbivory were 37% for exposed plants and 17% for protected plants. Among the exposed plants, high tannin concentration and tough leaves reduced herbivory. Twenty months after planting, the protected plants had a 10-fold higher growth rate, and 5.5 times more leaf area and dry mass than the protected plants. Twenty-two percent of the protected plants flowered, whereas no exposed plants flowered. Within the protected treatment, both total tannin concentration and leaf toughness varied among clones, which suggests that these traits are heritable. Total tannin concentration, but not toughness, was negatively correlated with growth for protected plants, which implies a cost to producing tannins. High tannin concentration and tough leaves reduced herbivory, however, these benefits of defense were balanced by the costs such that there were no significant differences in growth among exposed plants. These results are consistent with the hypotheses that there are benefits to being defended, that defense can be costly, and that herbivores act as selective agents in the evolution of plant defense.

Key words: Barro Colorado Island; cost of defense; exclosures; field experiment; herbivory; Panama; plant defense; *Psychotria horizontalis*; Rubiaceae; tannin; toughness; tropics.

INTRODUCTION

Optimality theory asserts that resource allocation to plant defense is based on the trade-offs between the benefits and costs of reducing herbivory. General theories predict that plants should invest in defense until the benefit of investment becomes limited by costs (Janzen 1973, Feeny 1976, Rhoades and Cates 1976, Lubchenco and Gaines 1981, Coley et al. 1985). Theory also suggests that plants must pay a metabolic price for shunting limiting nutrients into defense rather than growth or reproduction (McKey 1979, Chew and Rodman 1979, Mooney and Gulmon 1982, Gulmon and Mooney 1986), and that they pay an additional opportunity cost when resources committed to defense are unavailable for future investment (Coley et al. 1985, Baldwin and Ohnmeiss 1994). Over the lifetime of the plant, allocation to defense may become a substantial component of a plant's resource budget (Bazzaz et al. 1987).

The cost of defense will influence the evolutionary outcome of plant–herbivore interactions. If there is a genetic basis for investment in defense, herbivores

should selectively eliminate those genotypes for which the cost/benefit ratio is unfavorable. The benefit per unit cost should be maximized at an intermediate level of investment (McKey 1984, Coley et al. 1985, Fagerström et al. 1987, Simms and Rausher 1987, Rausher and Simms 1989), and an optimal defense should evolve. Where there are no costs, gain per unit of investment will be unchecked and directional selection will continue until all individuals are maximally protected, all other things being equal (Ehrlich and Raven 1965, Simms 1992).

Practical difficulties arise in choosing a currency with which to measure costs objectively (Chapin 1989, Simms 1992). Metabolic (Mooney 1972, Williams et al. 1987, Chapin 1989, Evans and Black 1993) and fitness currencies (Coley 1986, Simms and Rausher 1987) each have their advantages. Metabolic currencies reflect the physiological trade-offs involved in plant defense, but it is difficult to express benefits in these same units (Zangerl and Bazzaz 1992). A fitness currency more effectively reflects the evolutionary trade-offs between benefits and costs (Coley 1986), but lifetime fitness is often difficult to measure reliably, and must be estimated from its components. Nonetheless, the response of components of fitness to selection may provide a partial understanding of the mechanisms of evolution.

Fitness costs for defensive traits have been demon-

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strated in a variety of plant species (Chaplin 1970, Crawley 1985, Coley 1986, Loehle and Namkoong 1987), and have been found to vary among individuals within a species (Hanover 1966, Tester 1977, Edmunds and Alstad 1978, Marquis 1984, Berenbaum et al. 1986, Zangerl and Berenbaum 1990). Such results suggest that cost may be genetically variable and subject to selection. However, recent studies have had limited success in demonstrating genetic variation in the cost of defense, leaving in question the importance of costs in determining defense investment (Gould 1983, Simms and Rausher 1987, 1989, Brown 1988, Rausher and Simms 1989, Baldwin et al. 1990).

We evaluated both the benefits and costs to components of fitness in a series of common garden experiments. Benefits and costs were examined by manipulating herbivore densities on clones of *Psychotria horizontalis* Sw. (Rubiaceae), a design suggested, in part, by Marquis (1984), Berenbaum et al. (1986), and Simms and Rausher (1987). The impact of herbivory was evaluated for plants growing in the absence of herbivores compared to members of the same clone growing exposed to herbivores. The benefits of defense were assessed by comparing herbivory of exposed clones that differed in defense traits. The cost of defense was measured by the correlation between defensive traits and fitness. If resources are limited and defense is costly, plants that invest heavily in defense should have higher fitness in the presence of herbivores, but reduced fitness in the absence of herbivores. Also, since genetic variability is required for defenses to evolve, we compared familial correlations among clones for investment in defensive traits.

METHODS

Study site

We conducted this research on Barro Colorado Island (BCI) (9°09' N, 79°51' W), within the Barro Colorado Nature Monument, Republic of Panama (described by Leigh et al. 1982 and Gentry 1990). The climate is seasonal with heavy rainfall (2600 mm) from May to mid-December, and occasional rainfall (88 mm) during the remaining months (Rand and Rand 1982). The average annual temperature is 27°C, with the lowest temperatures occurring in the forest understory during the wet season and the highest temperatures occurring in the laboratory clearing during the dry season (Croat 1978). The average annual potential evapotranspiration is 146.4 cm (Dietrich et al. 1982). The soils of BCI are primarily well-weathered oxisols, poor in nitrogen, phosphorus, and potassium (Leigh and Wright 1990). The natural vegetation is considered tropical moist forest (Holdridge et al. 1971) or semideciduous moist forest (Foster and Brokaw 1982). No major disturbances have occurred in this century, but the eastern one-third of the island lay deforested until 1905, and small por-

tions of the island were cleared for farming as late as 1923 (Croat 1978).

Light is probably the most important abiotic factor influencing plant performance in this forest (Chazdon 1988). Light gaps form most frequently during the wet season and the largest gaps form in mature forest (Brokaw 1982). The mean time between treefalls at a given spot on BCI is 114 yr in mature forest and 159 yr in young forest (Brokaw 1982). Plants growing in large light gaps may receive 80% of full sun, whereas plants in the understory receive <2% (Chazdon and Fetcher 1984). In addition, temperature, soil moisture, and wind speeds are elevated in light gaps, and the relative humidity is reduced compared to that in the forest understory (Denslow et al. 1990). Herbivores may be more abundant in light gaps where primary productivity is higher (Coley 1983a, Aide and Zimmerman 1990, Marquis and Braker 1994). As a consequence, growth and reproduction of tropical forest species are highly dependent on light gap dynamics (Denslow et al. 1990, Sagers 1993a).

Study species

The genus *Psychotria* (Rubiaceae) consists of over 1600 species that are distributed throughout the tropics (Hamilton 1989). *Psychotria horizontalis* is an abundant, shade-tolerant shrub (Croat 1978) that may live up to 100 yr (R. Foster, *personal communication*). The population produces heterostylous flowers early in the wet season (May), and matures fleshy berries through November. Plants tend to produce a flush of leaves synchronously early in the wet season and continue producing leaves throughout the year at a much slower rate (Aide 1993). Rates of herbivory are high compared to other understory shrubs, and especially so in light gaps (C. L. Sagers, *personal observation*). *Psychotria horizontalis* hosts a large number of leaf-feeding herbivores, but the most damage is caused by two caterpillars, a pyralid and a ctenuchid (A. Aiello, *personal communication*), which can devour entire shrubs (Sagers 1992). *Psychotria horizontalis* can be propagated easily in the greenhouse from stems, leaves, or leaf fragments and it does so commonly in the forest as well (Sagers 1993b).

Psychotria horizontalis produces few secondary compounds. We found condensed and hydrolyzable tannins, but no alkaloids, saponins, cyanogenic glycosides, or terpenes (C. L. Sagers, *unpublished data*). Consumption of tannin by herbivores generally is associated with depressed growth rate and reduced efficiency of food utilization (Hagerman and Butler 1991), but the mechanism of tannin action is not completely understood.

Common gardens

The gardens were established to examine the phenotypic and genotypic correlations related to benefits and costs of defense. On 11 September 1989 (late wet

season), we cut 12 shoots tips from each of 36 individuals of *P. horizontalis* growing in the understory. Individuals were separated by >100 m to minimize the chance of sampling within a natural clone. Leaves were removed from each cutting and the stems were weighed and tagged. Initial stem mass was standardized among genotypes ($F_{35,396} = 1.23$, $P > 0.05$). Cut ends were treated with a fungicide/rooting hormone (Rootone) and placed in a moist, shaded sand bench in a screened growing house. After 8 wk (10 November 1989) cuttings were transplanted to the garden sites.

We chose three large light gaps, at least 250 m², in young forest along the Van Tyne and Barbour trails as our garden sites (Foster and Brokaw 1982). We did nothing to prepare the site besides remove dead, fallen tree limbs. We created an herbivore-free exclosure as the protected treatment by suspending fine mesh netting (11 × 1.5 × 1.5 m) in each light gap. The area was large enough to contain 72 cuttings. Cuttings in the exposed treatment were planted just outside of the exclosure. The top of the mesh exclosure was extended an additional 1.5 m from either side to ensure equal shading for exposed and protected cuttings. Cuttings from each clone were paired by size on the transplant day, and assigned randomly to a position within each of the three light gap gardens. Two pairs of cuttings from each clone were planted into each garden, one cutting of a pair was planted in the protected area, and the other in the exposed area 30 cm away.

We measured light availability, temperature, and relative humidity inside and outside of the exclosure. Light availability was measured at 10 points inside and outside of the exclosure with a LI-COR LI-190SA quantum sensor (LI-COR, Lincoln, Nebraska, USA) attached to a LI-189 quantum radiometer photometer. Relative humidity was measured with a sling psychrometer at five points, and temperature was measured at three points, inside and outside of each exclosure in each garden.

Plant defense

We evaluated three potential defenses of *P. horizontalis*: total tannin, condensed tannin, and leaf toughness. All assays were run on 6-wk-old, fully expanded leaves. In April 1991, we marked expanding leaf buds in the field with a twist of colored wire around the stem below the bud. Leaves were harvested 6 wk later for tannin and toughness assays. Leaves were collected into plastic bags and stored in a cooler for <1 h before extraction. One half of each fresh leaf was tested for toughness, and then weighed, oven dried at 60°C for 72 h, and reweighed to measure water content. The other half of the leaf was extracted by grinding in 80% methanol with a Polytron tissue homogenizer (Brinkman, New York, New York, USA). The homogenate was centrifuged at high speed in a bench-top centrifuge before analysis.

We used a protein-binding assay to characterize total

tannin concentration (Hagerman 1987), and a BuOH-proanthocyanidin procedure to measure condensed tannin (Mole and Waterman 1987). For the protein-binding assay, 40 µl of plant extract were placed in a well on a petri plate containing a mixture of agar and protein. Tannin in the extract bound the protein and formed an area of opaque precipitate that was proportional to the tannin concentration of the extract (Hagerman 1987). We used a tannic acid standard (Sigma Chemical Company, St. Louis, Missouri, USA, Lot number 87H0268) and expressed results in units of milligrams per gram TAE (tannic acid equivalents). For the condensed tannin measurement we used the BuOH-proanthocyanidin assay (Swain and Hillis 1959) with a quebracho tannin standard (P. Coley, personal supply) and expressed results in units of milligrams per gram QTE (quebracho tannin equivalents). The difficulty associated with choosing an appropriate tannin standard is minimal since the comparisons of interest are within a species (Mole and Waterman 1987).

Tannin production is induced in some plant species by damage to leaves (Feeny 1970, Karban and Myers 1989), but does not appear to be induced in *P. horizontalis*. Tannin concentration did not differ significantly between five undamaged and five artificially damaged individuals 2, 24, 96, or 720 h after damage (Kolmogorov-Smirnov two-sample tests, $N = 10$, $P > 0.10$ in each case).

Psychotria horizontalis has no hairs or spines, but leaf toughness could be a defense as it is in many tropical species (Coley 1983b). Leaf toughness was quantified by sheer strength, measured with a Chatillon push-pull gauge (Master Gauge Company, Chicago, Illinois, USA). This gauge records the mass necessary to force a 3 mm diameter rod through a leaf blade. Each leaf was perforated 10 times, and the mean for each leaf was used in statistical analyses.

Herbivory was measured as the proportion of leaf area removed from the first leaf flush in May 1990 until 12 mo later. Leaf area and area of damage were measured with an acrylic sheet scored with a 0.25-cm² grid. When an entire leaf was missing at a node, the missing leaf area was approximated by that of the opposite leaf. The rate of herbivory was calculated by dividing the total area removed by the estimated total leaf area produced during 12 mo. This estimate averages damage across all seasons for all leaves produced and is equivalent to an annual rate of loss to herbivory. For statistical analysis, these percentages were transformed as: $\ln [(\% \text{ leaf area lost over 12 mo} \cdot 1000) + 1]$ (Coley 1983b).

Benefits and costs of resistance

Several components of fitness were measured during the experiment. These include growth, flower number, leaf production, and final biomass. Growth among similar-sized plants was a good estimator of fitness. Larger plants had a higher probability of surviving (Kolmo-

TABLE 1. Analysis of variance results for the effects of garden, clone, and treatment on herbivory, survival, and relative growth rate. Mean square errors are from Type III sums of squares (SAS 1985). Herbivory = $\ln[(\% \text{ leaf area lost over 12 mo} \cdot 1000) + 1]$ (Coley 1983b); survival = $2 \arcsin[\sqrt{(\% \text{ survival by genotype})}]$ (Neter et al. 1985); relative growth rate = $[(\ln \text{ final dry mass}) - (\ln \text{ initial dry mass})]/\text{number of days}$ (Chiariello et al. 1989).

Source	Herbivory (%)			Survival (%)			Relative growth rate (g/d)		
	df	MS	F	df	MS	F	df	MS	F
Garden	2	28.31	23.57****	2	5.68	4.99**	2	4.25	2.28
Clone	35	0.88	0.73	35	1.68	1.47	35	2.28	1.22
Treatment	1	23.12	19.25****	1	0.28	0.25	1	298.00	159.69****
Treatment \times clone	34	0.62	0.52	34	1.14	1.00	34	2.20	1.17
Error	143	1.20		215	0.11		258	1.87	

** $P < 0.01$, **** $P < 0.0001$.

gorov-Smirnov two-sample test, $N = 245$, $P < 0.05$). Also, plant growth and size generally correlate with the number of reproductive meristems (Bloom et al. 1985, Samson and Werk 1986). The relative growth rate from planting date to harvest date was measured as: $[(\ln \text{ initial mass}) - (\ln \text{ final mass})]/\text{number of days}$

(Chiariello et al. 1989). Plant size was measured as the change in leaf area, and change in biomass, and all measures were log transformed for analysis. Survival was estimated for each clone as the percent of stems still living on the harvest date. Survival rates were transformed as: survival = $2 \arcsin(\sqrt{\%})$ (Neter et al. 1985). All plants were harvested 20 mo after planting (May–June 1991).

The impact of herbivory was measured by comparing the performance of plants in the protected and exposed treatments. Since there is no evidence for induction in defense, the critical difference between treatments was the result of experimentally reducing herbivory. The heritable variation in defense investment was assessed by comparing defense levels among different clones within a treatment. The benefits of defense investment were evaluated by comparing rates of herbivory for exposed clones with different levels of defense. A negative correlation between defense traits and herbivory would suggest a benefit to defense. The cost of defense was evaluated for plants within the protected area by examining the correlation between the change in dry mass (biomass) and the measured level of defense. A negative association between biomass and levels of defense would be consistent with the hypothesis of a cost to defense. A similar comparison was repeated for exposed plants to evaluate the variation among individuals in the balance between benefits and costs.

Analyses

All statistical comparisons were made using the General Linear Models procedure of the Statistical Analysis System (SAS 1985). The variation in herbivory, defensive traits, and fitness components among clones was analyzed with ANOVA as a randomized block design (Neter et al. 1985).

We used partial correlations between the properties of leaves (total tannin, condensed tannin, or toughness) and herbivory to determine which traits were associated with reduced herbivory, and plant growth. Partial correlations measure the marginal contribution of a variable when all other variables are already included in the model (Neter et al. 1985). A partial correlation is

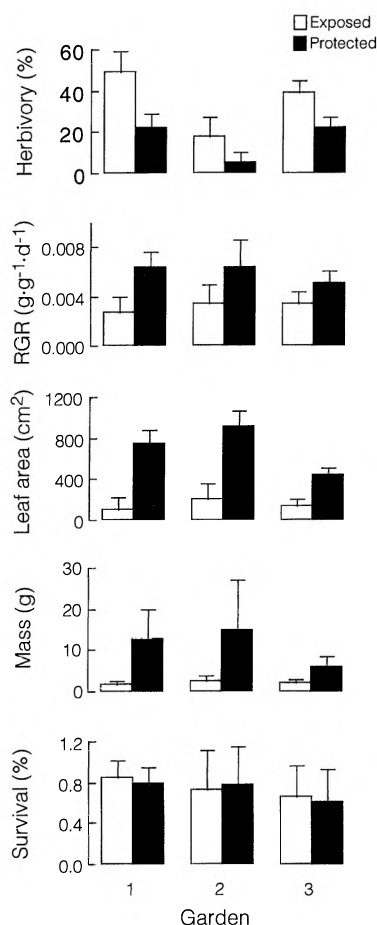


FIG. 1. Effects of herbivory treatment on mean herbivory, survival, relative growth rate, final leaf area, and dry mass in three experimental gardens. Protected plants (solid bars) were grown within a mesh cage. Exposed plants (open bars) were grown outside of the cage. Error bars represent 1 SE of the mean.

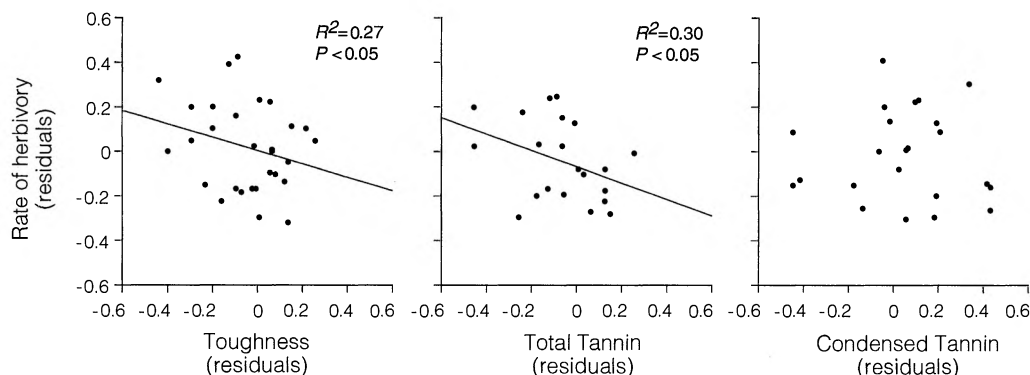


FIG. 2. Partial correlations of herbivory rate and leaf traits for plants exposed to herbivores. Partial correlations are calculated from the residuals of a multiple regression of toughness, or total tannin, or condensed tannin on herbivory. The residuals are the variation not explained when the variable of interest is not included in the model.

calculated from the residuals that remain after the initial regression.

Genetic variance was approximated by the analysis of differences among clones rather than sibships in this study. Some caution in the genetic interpretation is warranted since the resemblance of cuttings within a clone may be affected by maternal effects in addition to differences in nuclear DNA (Libby and Jund 1962).

RESULTS

Impact of herbivory

The exclosures reduced herbivory without affecting microclimate. Herbivory for the protected plants (17%) was significantly lower than for the exposed plants (37%) ($P < 0.001$) (Table 1, Fig. 1), but did not differ significantly among clones in either treatment. Light availability, relative humidity, and temperature did not differ significantly between the inside and outside of the exclosure (Kolmogorov-Smirnov two-sample test, $P > 0.05$ in each case).

Growth and reproduction differed significantly between the protected and exposed treatments (Table 1, Fig. 1). Relative growth rate was substantially higher in the protected treatment than in the exposed treatment (Table 1, Fig. 1). Measures of plant size that are highly correlated with relative growth rate (final leaf area: $R^2 = .92$, $P < 0.0001$, change in biomass: $R^2 = 0.97$, $P < 0.0001$) showed similar responses to exposure to herbivory (Fig. 1). Further, 22% of the protected plants

flowered, but no exposed plants flowered. Growth and reproduction did not differ among clones in either treatment (Table 1).

Overall survivorship did not differ between treatments ($P > 0.05$), (Table 1, Fig. 1), nor among clones ($P > 0.05$) (Table 1). The pattern of mortality was bimodal with one peak (38%) in December 1989 at the beginning of the dry season, and a second peak (38%) in May 1990 at the end of the dry season, which suggests that most of the mortality was due to environmental stress.

Benefit of defenses

Toughness and tannins are both effective defenses as increased levels were associated with reduced herbivory. The partial correlations of toughness and total tannin with herbivory were negative and significant for plants in the exposed treatment (Fig. 2). Total tannin and toughness are not correlated ($P = 0.89$, $N = 215$) and therefore can be treated as independent traits. Furthermore, in feeding trials with *P. horizontalis*, pyralid caterpillars preferred tender leaves with low tannin concentrations (Sagers 1992). Condensed tannin appears to have no association with herbivory as there was no significant effect of including it in the regression model (Fig. 2).

Variation in defenses among clones

We used ANOVA to evaluate the effect of garden and clone on defense investment and found that the clones varied significantly for both total tannin and toughness (Table 2). We also found a significant interaction term between genotype and total tannin (Table 3), which suggests that the cost of investing in total tannin varies among clones. These differences among clones provide a basis to expect genetic variance for investment in defense.

Cost of defense

Growth, total tannin, and toughness varied for plants protected from herbivory. Because toughness and total

TABLE 2. Analysis of variance results for the effects of garden and clone on total tannin, and on toughness for plants protected from herbivory. Mean square errors are from Type III sums of squares (SAS 1985).

Source of variation	Total tannin			Toughness		
	df	MS	F	df	MS	F
Garden	2	0.04	0.51	2	0.08	4.1*
Clone	35	0.20	2.53*	35	0.04	2.26*
Error	172	0.08		172	0.02	

* $P < 0.05$.

TABLE 3. Analysis of variance results for the effects of garden, family, and leaf traits on relative growth rate for plants protected from, vs. exposed to, herbivory. Mean square errors are from Type III sums of squares (SAS 1985).

Source of variation	Protected			Exposed		
	df	MS	F	df	MS	F
Garden	2	29.41	12.9*	2	6.86	6.19**
Clone	32	13.91	6.1*	30	0.80	0.72
Total tannin	1	12.77	5.6*	1	1.81	1.63
Toughness	1	4.79	2.1	1	2.36	2.13
Total tannin \times toughness	1	3.65	1.6	1	3.4	3.07
Clone \times total tannin	32	3.92	1.72*	30	1.52	1.37
Clone \times toughness	32	3.35	1.47	30	1.00	0.91
Clone \times total tannin \times toughness	32	2.26	0.99	30	1.57	1.42
Error	76	2.28		82	1.11	

* $P < 0.05$, ** $P < 0.01$.

tannin are not correlated, they can be treated as independent variables in the analyses of variance. For plants protected from herbivory, relative growth rate and toughness were not correlated (Table 3, Fig. 3), suggesting there is no measurable growth cost associated with toughness. In contrast, we found a significant, negative, partial correlation between growth rate and total tannin (Table 3, Fig. 3). This negative relationship was clear after only 20 mo, a relatively brief period in the life of a long-lived, perennial plant.

Balancing benefits and costs

The combined consequences of the benefits and costs of defense can be evaluated from a comparison of growth among clones in the exposed treatment. Here, there is no significant effect of clone, total tannin, nor toughness on growth (Table 3), suggesting that the costs and benefits are equal among clones. Although growth in the exposed treatment is significantly lower than in the protected treatment, there is no significant relationship between growth and defense levels (Fig. 4). This again suggests that all plants have similar cost/benefit ratios. Therefore, under natural herbivory, the defense costs of producing tannins (Fig. 3) are balanced by the benefits of reduced herbivory (Fig. 2) such that fitness is constant across defense levels (Fig. 4). In contrast, protected clones showed a negative relationship between total tannin and growth (Fig. 3). Under natural exposure to herbivores, this defense cost of total

tannin is apparently balanced by the benefits of reduced herbivory (Fig. 2).

DISCUSSION

We have shown that herbivory has a significant influence on plant reproduction and growth. In this study, a twofold increase in herbivory was sufficient to delay reproduction, and reduce the growth rate by 40% (Table 1, Fig. 1). It would seem that reducing herbivory by investing in defense would be extremely beneficial. Clones differed significantly in defense investment, and clones with higher levels of total tannin and toughness were better protected from herbivory. However, investment in defense may be limited by costs. We have shown that investment in total tannin is associated with reduced growth for plants in the protected treatment. This relationship is not expressed in plants growing in the presence of natural levels of herbivory, suggesting that for *P. horizontalis* under high levels of herbivory, the costs of defense balance the benefits of reduced herbivory.

This study complements previous studies by demonstrating clonal variability in investment in total tannin, which suggests a heritable basis for this plant trait. Furthermore, tannin production and maintenance appear to be costly in terms of growth and reproduction. However, we found no trade-off between toughness and growth. This may be because: (1) growth and toughness are limited by different resources, or (2) there is no

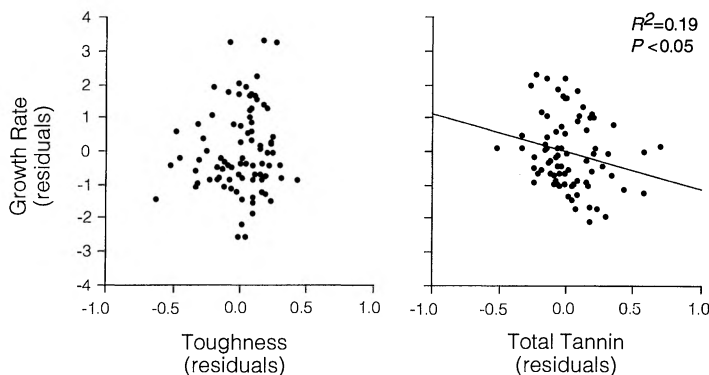


FIG. 3. Partial correlations of relative growth rate and defense for plants protected from herbivores. Partial correlations are calculated from the residuals of a multiple regression of toughness and total tannin on growth rate.

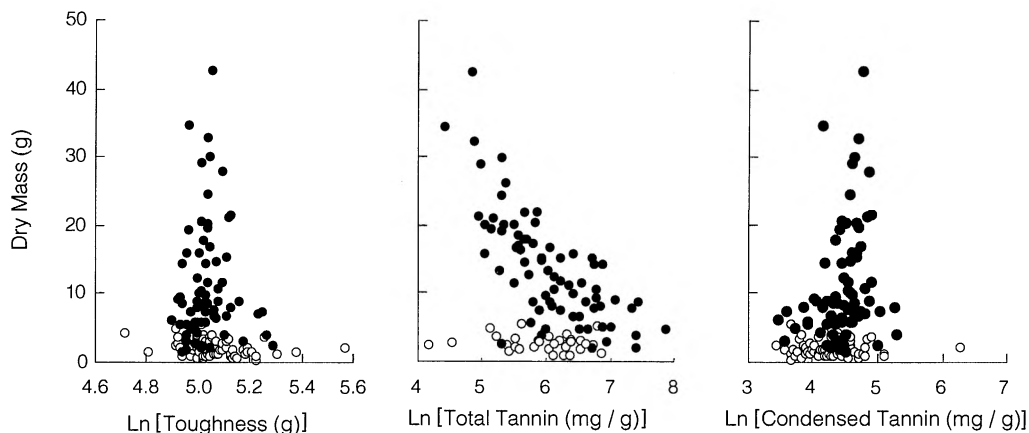


FIG. 4. Correlations between change in dry mass and mean level of toughness, total tannin, and condensed tannin for plants growing in the protected (●) and exposed (○) treatments.

cost of toughening leaves, or (3) selection has reduced the per unit cost so that it cannot be measured, or (4) toughness has auxiliary beneficial functions that reduce the cost of defense (Simms 1992). Leaves are toughened by fiber and lignin, metabolically expensive compounds (Gulmon and Mooney 1986) that have both defensive and structural properties (Swain 1979, Zucker 1983, Stafford 1988). Their structural benefit may preclude an additional cost for defensive function, thus preventing us from detecting an added, herbivory-related cost.

Given that plant defense was beneficial, total tannin was costly, and variation occurred among clones, selection on tannin should be stabilizing for an optimal level of defense. Selection can be characterized by the shape of the relationship between a character and fitness (Lande and Arnold 1983). Under stabilizing selection, we expect most genotypes in the population to have equal fitness. And this appears to be the case for *P. horizontalis* clones under natural conditions of herbivory. All clones exposed to herbivory had similar fitnesses (Table 3, Fig. 4). Small increases in defense costs appeared balanced by benefits associated with reduced herbivory. Presumably, genotypes with much higher or lower defense investments were at a selective disadvantage and either were eliminated from the population or were too rare to have been sampled.

The similarity of fitness components observed among clones under natural levels of herbivory could be due to the shape of the cost/benefit trade-off curve for defenses, as described above. Alternatively, genotypes may have similar fitness because of fluctuating selection: costs may vary spatially and temporally as resources and herbivores are more or less abundant (Gillespie and Turelli 1989). Spatial heterogeneity influenced defense investment in *P. horizontalis* as evidenced by the significant block effect of garden on defense investment (Table 3). Further, levels of herbivory dictated the magnitude of the benefit, as is clear

from a comparison of biomass for exposed and protected plants (Fig. 4). Temporal and spatial variability in the benefits and costs of defense may preclude optimizing selection (Gillespie and Turelli 1989).

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